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Intranasal administration delivers peptoids to the rat central nervous system

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ABSTRACT

Intranasal administration of therapeutic agents offers a noninvasive method of drug delivery that bypasses the blood–brain barrier and directly targets the central nervous system (CNS) and lymph nodes. We examined whether intranasal peptoid CHIR5585, an antagonist of the urokinase plasminogen activator receptor (uPAR), is delivered to the CNS. Peptoids are a novel class of peptide isomers that are oligomeric N-substituted glycine peptides. Anesthetized male rats were administered peptoid CHIR5585 intranasally, and tissue distribution was evaluated quantitatively by gamma counting and qualitatively by autoradiography. Intranasal administration resulted in significant delivery throughout the CNS (olfactory bulbs, 3.9 μM; cortex, 0.3 μM; trigeminal nerve, 1.7 μM) and deep cervical lymph nodes (4.5 μM). Autoradiography demonstrated a similar delivery pattern to the CNS.

The blood–brain barrier (BBB) interferes with the treatment of neurological diseases by many different therapeutic drugs; this includes many of the newer engineered drugs, such as antisense, gene vectors, proteins and peptoids. The BBB is comprised in part of tight junctions between the capillary endothelial cells in the brain microvasculature; this prevents free diffusion of molecules across the BBB and prevents most molecules from reaching the central nervous system (CNS) from the bloodstream. Often therapeutic compounds are designed and selected based in large part on their ability to cross the BBB rather than simply on their potential safety and efficacy. However, intranasal (IN) administration of drugs provides a method for bypassing the BBB and directly delivering therapeutic agents to the CNS while reducing systemic exposure and unwanted systemic side effects [6,7].

Drugs administered IN to the nasal cavity can travel along the olfactory and trigeminal nerves to reach many areas of the CNS [21]. A number of protein therapeutic agents have previously been delivered to the rodent CNS via the IN route [6,7]. Similarly in humans, IN administration of the peptide hormones, melanocortin, vasopressin, and insulin, are directly delivered into the cerebrospinal fluid of the CNS [3]. Intranasally administered drugs reach the CNS within minutes, and are thought to be transported extraneuronally [6,7,17,21].

In addition to drug distribution studies, IN delivery has been shown to treat neurological diseases in various animal models. Intranasal IGF-I reduces infarct volume and neurological deficits after stroke [12] while NGF decreases neuropathology [4] and increases memory [5] in a mouse model of Alzheimer's disease. Furthermore, intranasal IL-10 suppresses acute and protracted-relapsing experimental allergic encephalomyelitis—a model of multiple sclerosis [23], and methotrexate shrinks brain tumors [18]. In human studies, IN insulin improved mood and memory in normal adults [2], and administered acutely improved memory of humans suffering from Alzheimer's disease or mild cognitive impairment [16]. Intranasal insulin did not alter plasma insulin or glucose levels [16], indicating a delivery method to target the CNS and avoid peripheral side effects of the administered drug. Intranasal administration provides a simple, practical, and rapid route for drug delivery to the CNS.

The objective of this study was to determine whether IN administration of a novel class of peptide isomers, peptoids, are delivered to the CNS as previously shown for peptides. Peptoids are oligomeric N-substituted glycines that are easy to synthesize [19] resistant to proteolytic enzymes [13] and have potent biological activity. Peptoid synthesis can provide a chemically diverse library with limitless diversity of molecular structure that may act as either receptor agonists or receptor antagonists. Although peptoids have increased stability, there is evidence that peptoids have decreased oral absorption compared to natural peptides [22], and only a small percentage (0.2%) of intraperitoneally administrated peptoid has

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Table 1
CHIR5585 concentrations (nM) in CNS tissues following IN administration

	Mean	S.E.
Olfactory bulbs	3934	455
Anterior olfactory nucleus	674	177
Frontal cortex	297	68
Caudate/putamen	135	26
Septal nucleus	415	94
Hippocampus	165	60
Diencephalon	199	66
Brain stem	234	100
Cervical spinal cord	178	55
Thoracic spinal cord	77	10
Lumbar spinal cord	79	12

Table 2
CHIR5585 concentrations (nM) in peripheral tissues following IN administration

	Mean	S.E.
5 min blood sample	685	177
10 min blood sample	1234	147
15 min blood sample	2254	116
20 min blood sample	3301	365
25 min blood sample	3517	331
Olfactory epithelium	90878	31622
Dura mater	3255	1527
Optic nerve	1341	474
Trigeminal nerve	1732	477
Common carotids	1025	320
Superficial cervical lymph node	942	220
Deep cervical lymph nodes	4484	1102
Axillary lymph nodes	423	105
Liver	567	42
Kidney	2613	780
Spleen	303	54
Deltoid muscle	322	67

been previously demonstrated to cross the BBB [1]. To circumvent this problem, we examined the ability of IN administration to deliver peptoid CHIR5585 [25] to the brain and spinal cord. Peptoid CHIR5585 is an antagonist of the urokinase plasminogen activator receptor (uPAR), a major determinant of transformation and metastatic potential of glioblastoma. Peptide antagonists or antisense downregulation of uPAR have been shown to have anti-tumor, anti-invasive and anti-angiogenic effects in glioblastoma [9,14], which suggest the need for direct delivery of CHIR5585 to the brain. This study determined the biodistribution of IN administration of CHIR5585 to the CNS and surrounding structures.

Five male Harlan Sprague–Dawley rats (221–246 g) were anesthetized with intraperitoneal sodium pentobarbital (50 mg/kg) to prevent any pain or discomfort. After cannulating the descending aorta, the rat was placed on its back with its neck slightly elevated, and body temperature maintained with a heating pad and a rectal thermal probe (Fine Science Tools) set at 37 °C. Peptoid (molecular weight: 707.8) was delivered as a mixture of unlabeled CHIR5585 (Chiron) and ^{125}I-labelled CHIR5585 (Amersham (now called GE Life Science Products). The unlabeled CHIR5585 was used as a carrier molecule and to provide a significant molar dose, and the ^{125}I-labelled CHIR5585 was used as a tracer molecule to follow the movement of CHIR5585. Peptoid (total concentration of 4.8 mg/ml and 1.3 mCi/ml) was IN administered with a Pipetteman pipetter to alternating nares every 2–3 min in 4–10 μl drops. Over 25 min, a total volume of 68 μl of CHIR5585 was administered IN. A 0.2 ml blood sample was collected from the descending aorta cannula approximately every 5 min.

Approximately 30 min after onset of IN administration, the rat was perfused through the descending aorta cannula with 75 ml of 0.9% NaCl followed by 400 ml of fixative (4% paraformaldehyde in phosphate buffer). For quantitative biodistribution studies ($N = 3$ for CNS tissue in Table 1 and $N = 5$ for nerve and meninges tissue in Table 2; $N = 5$ for peripheral tissue in Table 2), peripheral and CNS tissues were dissected into individual sections and placed in a 5-ml Sarstedt tube for gamma ray counting in a Packard Cobra II Auto-Gamma counter. For autoradiography ($N = 2$), 1-mm brain sections were placed on glass microscope slides, covered with plastic wrap, and set onto a phosphor screen for 21 days in an autoradiography cassette. The screen was developed in a Cyclone Phosphor Scanner (Packard), and data were analyzed with Packard Optiquant software.

Biodistribution studies demonstrated that IN administration of 464 nmol (81 μCi) CHIR5585 resulted in significant delivery throughout the CNS (Table 1) and cranial nerves (Table 2). Approximately 200 nM tissue concentrations were found throughout the brain (i.e., cortex, caudate putamen, hippocampus, brain stem, cerebellum), which is similar to the peptoid IC50 of 225 nM for uPAR. The highest CNS tissue concentration was found in the olfactory bulbs (3934 nM), followed by the trigeminal nerve (1732 nM), optic nerve (1341 nM), anterior olfactory nucleus (674 nM), and septal nucleus (415 nM) (Tables 1 and 2). In the spinal cord, IN CHIR5585 resulted in a decreasing concentration gradient along the spinal cord from cervical to thoracic to lumbar regions (Table 1). Entry via the trigeminal nerve (Table 2) into the brainstem near the cervical spinal cord is probably responsible for the high cervical spinal cord concentration.

Intranasal CHIR5585 resulted in high delivery to the deep cervical lymph nodes with much lower delivery to the superficial cervical lymph nodes and axillary lymph nodes (Table 2). Intranasal CHIR5585 also resulted in distribution to the blood and peripheral organs (Table 2). The blood concentrations of CHIR5585 increased over time with a final concentration of 3517 nM at 25 min after the beginning of IN administration.

Autoradiographic analysis was used to confirm the quantitative biodistribution study and to obtain greater detail of CHIR5585 distribution in the brain. In agreement with the quantitative biodistribution studies, autoradiographic screens demonstrate IN delivery of CHIR5585 (425 nmol, 78 μCi) resulted in drug distributions throughout the entire brain (Fig. 1). Greater drug distribution occurred in the rostral half of the brain compared to the caudal half of the brain (Fig. 1A), with greatest drug delivery to the olfactory bulbs. Detailed analysis demonstrates delivery to the septal nucleus, caudate putamen, and olfactory tract (Fig. 1B); and caudal brain sections showed delivery to hippocampus, thalamus, and hypothalamus (Fig. 1C). Autoradiography also showed delivery of peptoid throughout the entire cortex.

The high concentration of CHIR5585 in the olfactory bulb (3934 nM) and trigeminal nerve (1732 nM) suggest that the drug enters the brain along these nerves as previously demonstrated with other peptides, such as IGF-I [21] and IFNβ [17]. Both the olfactory nerve and trigeminal nerve innervate the nasal cavity and provide intraneuronal and extraneuronal pathways into the brain [21]. Drugs traveling along the extraneuronal pathway can reach the brain within minutes and account for the results presented here since intracellular delivery requires hours to days to reach most brain structures [6,11]. High concentrations were observed in the dura mater (3255 nM), which suggests that CHIR5585 may also be traveling through the meninges or its associated perivascular spaces to enter various brain tissues. Intranasal administration also resulted in drainage to the lymphatic system as observed with the delivery to the lymph nodes (942 nM for superior cervical nodes, and 4484 nM for deep cervical nodes); this has also been observed with IN IGF-I [21] and IFNβ [17].

Intranasal administration of CHIR5585 results in distribution of peptoid throughout the brain, spinal cord, trigeminal nerve, and

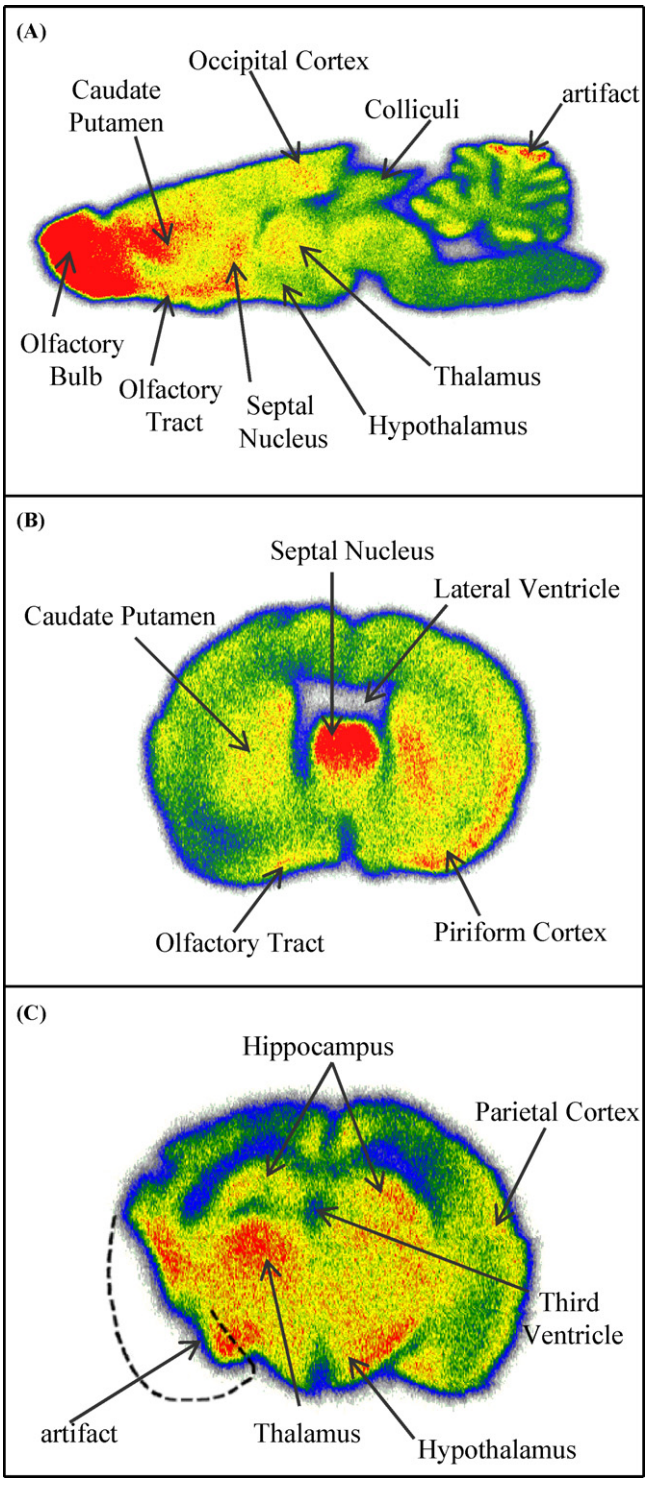

Fig. 1. Autoradiography following intranasal delivery of CHIR5585. Anesthetized rats were IN delivered 425 nmol and 78 μCi as of ^{125}I-labeled CHIR5585 + unlabeled CHIR5585 mixture. Twenty-seven minutes after onset of delivery, the fixed brain was sliced into 1-mm sections and incubated on a cyclone phosphor screen for 21 days. (A) Sagittal section corresponding to about lateral 1.0–2.0 mm. (B) Coronal section corresponding to ~Bregma −0.4 mm. (C) Coronal section corresponding to ~Bregma −3.14 mm.

meninges. Having demonstrated the biodistribution of CHIR5585, future studies could examine the therapeutic efficacy of CHIR5585, a uPAR antagonist, in the treatment of glioblastomas. Brain tumor size has been successfully reduced with intranasal methotrexate [18]. Several studies have demonstrated potent efficacy of peptoids. A noncompetitive VR1 antagonist peptoid attenuated thermal and inflammatory nociception [8]. The peptoid CHIR29498 displays rapid bactericidal activities against a panel of gram-positive and gram-negative bacteria and protected S. aureus-infected mice [10]. A peptoid delivered to mice attenuated NMDA receptor-induced cell death and reduced neurodegeneration in stratum in rat cerebral ischemia [15]. Lastly, a melanocortin peptoid agonist altered rat grooming behavior [11]. Intranasal melanocortin peptide is delivered to the CSF in humans [3]. Intranasal administration of the melanocortin peptoids may provide a more stable method for treating obesity.

Intranasal administration provides a method to directly deliver peptoids to the CNS. Using the IN method of drug delivery does not require any chemical modification of the therapeutic agent to enhance a drug's BBB permeability; because IN drugs are able to bypass the BBB and be directly delivered to the CNS. This route of delivery will avoid some of the disadvantages seen with oral administration of peptoids, producing an efficacious dose in the CNS while decreasing exposure of peripheral organs to the drug. Past studies have shown IN delivery results in significantly lower systemic exposure compared to intravenous administration of peptides that results in significantly higher delivery of peptides to peripheral organs [7,17,21]. A higher concentration of CHIR5585 was observed in the kidneys (2613 nM) compared to other peripheral organs, as was similarly seen previously with interferon beta-1β [17]. This suggests that the kidneys play a role in elimination of the CHIR5585 peptoid as with other proteins. The concentration of CHIR5585 found in the kidneys was still lower than that measured in the blood, olfactory bulb, and meninges.

This study demonstrates a new route of drug delivery for the peptoid class of drugs that bypasses the BBB and produces significant concentration in the CNS. Intranasal deliver of the uPAR antagonist, CHIR5585, along with other peptoid agonists or antagonist of 7 transmembrane receptors [24] or alteration of enzymatic activity, such as inhibition of unwanted proteolysis [20] could be used in a variety of neurological disorders. Intranasal administration of therapeutic compounds provides a method of targeting the CNS while keeping systemic exposure at a minimum, thus improving the treatment of neurological diseases while reducing unwanted systemic side effects.

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